

The University of Chicago

FACTORS INFLUENCING THE ASEXUAL
PERIODICITY OF AVIAN MALARIAS

A PART OF A DISSERTATION SUBMITTED
TO THE FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY.

DEPARTMENT OF BACTERIOLOGY AND PARASITOLOGY
1937

By
LESLIE ALFRED STAUBER

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LESLIE A. STAUBER²

INTRODUCTION

The mechanism underlying the length of the asexual cycle and the synchronism of reproduction is of basic importance in obtaining not only a better knowledge of the malaria parasites, but of the host reaction as well. The diversity of periodic rhythms among the malaria parasites, however, complicates the problem. Among the avian parasites alone, there are species or strains which have little or no periodicity. Others have cycles lasting 48 hours (Huff, 1935; Wolfson, 1936b), 36 hours (Gingrich, 1929), 30 hours (L. G. Taliaferro, 1925), 24 hours (L. G. Taliaferro, 1925; Huff and Bloom, 1935; Huff, 1937; Wolfson, 1936a, 1937), or 12 hours (Bovet, 1930). Some segment in the morning hours while others segment during the evening hours. W. H. Taliaferro (1932) suggested that the length of the asexual cycle of each malaria infection is largely determined by the genetical constitution of the parasite, but that the time of segmentation and the synchronism of reproduction are largely determined by the host. If only the host factor were involved, all parasites would have quotidian infections. If the host played no part, each strain might be expected to have a different type of infection. Most of the strains of malaria, however, have synchronous rhythms which are multiples of 24 hours.

If the part the host plays be largely of the nature of a consistent but cyclically changing culture medium, alteration of this culture medium should lead to a better knowledge of the factors controlling the synchronism and time of segmentation. Changes in temperature, light, feeding, host activity and rest were accordingly used in this study of the

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periodicity of the asexual rhythm. Of these, environmental temperature and the period of rest or sleep were measurably effective. It was demonstrated that light acted by compelling the host to activity—a result produced through the eyes as distinguished from a body surface effect. In the life cycle of the parasite the young trophozoite seemed to be the stage most susceptible to these influences. In the host the diurnal fluctuation of body temperature or a closely related phenomenon appeared to be responsible for the synchronism and time of appearance of asexual reproduction.

HISTORICAL REVIEW

Golgi first reported on the asexual cycles of the malaria parasites of man and related them to the familiar tertian and quartan fevers. In 1925, L. G. Taliaferro demonstrated that *Plasmodium cathemerium*, in the canary, regularly and synchronously reproduces every 24 hours with schizogony occurring largely from 6–9 P. M. These original observations have been abundantly confirmed for *P. cathemerium* by Drensky and Hegner (1926), Hartman (1927), G. H. Boyd (1929a and b, and with Allen in 1934), Huff and Gambrell (1934), Lourie (1934) and by the work reported here. The work has been extended in recent years, especially by Huff and also Wolfson, to include studies of the periodicity of many of the growing list of bird malaria parasites inoculable into the canary.

This striking periodicity of reproduction naturally led to speculations as to its nature and mechanism. Is it an inherent property of the parasite or is it conditioned by the host? In 1928, L. G. Taliaferro showed that by *in vitro* refrigeration for 6 or 12 hours reproduction of *P. cathemerium* could be delayed by an interval equal to the time of refrigeration, but that it soon returned to normal when the refrigerated parasites were inoculated into a normal canary. Boyd (1929a) by reversing the light and dark schedule, obtained a reversal of the periodicity of the asexual reproduction of *P. cathemerium*. It is to be noted that the time of segmentation and not the length of the asexual cycle was changed. W. H. and L. G. Taliaferro (1934b) obtained a similar result with the quartan malaria parasite, *P. brasilianum*. The simian parasite, however, not only shifted its peaks of segmenting parasites, but also split into two broods, one segmenting 12 hours previous to and the other 12 hours subsequent to the original time of segmentation. Gambrell (1937), by a similar procedure, was able to reverse the time at which the peaks of gametocytes as well as of segmenting asexual parasites appeared. She used the "D" strain of *P. cathemerium* and the "R" strain of *P. relictum* var. *matutinum* in both of which the periodic production of gametocytes had been determined. In another paper Boyd (1929b) found that alter-

nate light and dark periods of 6, 8 or 16 hours produced no effect on the 24 hour periodicity, but that alternate light and dark periods of 14 or 20 hours disturbed the rhythm and that prolonged exposure to an experimental day and night of 14 hours each changed the periodicity from 24 to 28 hours. Gambrell (1937) found that, when canaries were subjected to alternate 24-hour periods of light and darkness, gametocyte production became asynchronous concomitantly with the loss of the periodic asexual rhythm. In 1933 Boyd seemed to eliminate host fatigue as a factor in influencing the reproductive activity of the parasite but suggested that "factors associated with host feeding seem to exert considerable influence upon the reproductive activity of this parasite." Huff and Gambrell (1934) found that races of *P. cathemerium* ("D" strain) not producing gametocytes did not show a periodic rhythm of asexual reproduction. Gambrell (1937) obtained from the "N" strain also a series of strains of parasites completely lacking in gametocytes and in periodicity of the asexual forms.

Other modifications of the synchronism or periodicity of the malaria parasite have been attempted. For example, L. G. Taliaferro (1925) reported that in *P. cathemerium*, in one case at least, the parasites of the first and second relapses did not show as high a degree of synchronism as in either the acute or chronic periods. Since adrenalin chloride was used to initiate the relapses we have a possible effect of this drug on the periodicity of the parasites. Boyd and Allen (1934) learned that quinine hydrochloride administered daily to infected canaries retarded the rate of growth of the parasite, influenced the adult size and delayed the time of the asexual cycle. Lourie, in 1934, in a more extensive publication on the mode of action of quinine independently came to similar conclusions. Wolfson (1936b) noted an apparent disturbance in the regularity of the periodicity of the avian tertian parasite (*P. circumflexum*) after a large amount of blood had been removed from the bird. In their extensive study of a simian malaria parasite, W. H. and L. G. Taliaferro (1932, 1934a, b and c) observed several modifications of the synchronism and length of the asexual cycle. They noted that if the parasites were refrigerated *in vitro* for several hours before injection into an uninfected monkey, or occasionally under ordinary manipulations, the first cycle took 4 days, and that both the synchronism and length of the asexual cycle of the parasites were disturbed occasionally during the crisis of the acute infections and also of the superinfection due to the immune response of the host. In all the above cases, however, when the abnormal factor was no longer acting (quinine, manipulation, refrigeration, etc.) the normal rhythm quickly returned although with the tertian and quartan parasites the peaks of segmentation might be a day or two later than anticipated.

MATERIALS AND METHODS

The female canary, *Serinus canarius*, was used throughout this work. One of the toe veins was used for all intravenous injections of parasites and blood for smears was taken from a leg vein. To study the parasites, blood smears were generally made at 2- or 4-hour intervals over a period of 72 (sometimes 24) hours and were air dried, fixed in absolute methyl alcohol and stained with Mac Neal's tetrachrome, prepared according to Gingrich's modification (1932).

The following three strains of *P. cathemerium* and one strain of *P. relictum* var. *matutinum* were used: "C" strain, *Plasmodium cathemerium*, isolated from a sparrow by Dr. Ernest Hartman in 1924, and used by L. G. Taliaferro (1925 and 1928) and G. H. Boyd (1929a and b, 1933 and 1934); "N" strain, derived from a single parasite of *P. cathemerium*; "D" strain, *P. cathemerium*, isolated from a sparrow by Dr. Hackett in Italy, shipped to Dr. W. Gingrich in mosquitoes and used by Huff (1934) for numerous infectivity experiments; "R" strain, *P. relictum* var. *matutinum*, isolated from a robin by Huff (1937).

The "C" "D" and "N" strains of *P. cathemerium* are similar except that the "D" and "N" strains occasionally tend to produce strains that lack gametocytes and periodicity. The periodicity is synchronous with the peak in segmentation occurring in the evening hours under animal room conditions or at the beginning of the dark period under more rigidly controlled conditions (see L. G. Taliaferro, 1925, and others for the "C" strain; Huff and Gambrell, 1934, and Gambrell, 1937, for the "D" and "N" strains). The periodicity of the "R" strain of *P. relictum* var. *matutinum* is extremely synchronous with the peak in segmentation occurring in the morning hours or at the beginning of the light period. Since the periodicity of this strain is so synchronous, it would have been particularly suitable for use in this work, but unfortunately infections were often very low grade and transitory.

To regulate the light and dark periods, a cabinet containing four light-tight compartments with separate light sources and time switches (Fig. 1) was used. Birds were placed in standard wire bird cages within a compartment. The illumination at the approximate center of these cages was measured by means of a Weston or Westinghouse light meter, a photoelectric cell with the ammeter graduated in foot candles of light. Control birds were always supplied with a light source equivalent to conditions better than the poorest in the animal room on a cloudy day. Where the light might have been of such an intensity as to interfere with the usual air temperature of a compartment, water filters were used.

The method used by W. H. and L. G. Taliaferro (1934a) to study periodicity was adopted in this work. It involves ascertaining the percentage of segmenters in a random sample of parasites on stained blood

films at close intervals for a sufficient time during the course of a malarial infection. Thus, in a strain showing periodicity, the percentage of segmenters will be none or very few for a certain number of hours and then will increase to a peak and decrease as sporulation occurs. The method is adequate provided it is modified to fit the characteristics of a given periodicity. For example, smears will need to be made more often to show a periodicity of 24 hours than to show one of 72 hours; a segmenter will need to be considered as a parasite with 5 or more nuclei if the completed segmenter forms very few merozoites; and care has to be taken to make blood films within the sporulation period. (In my

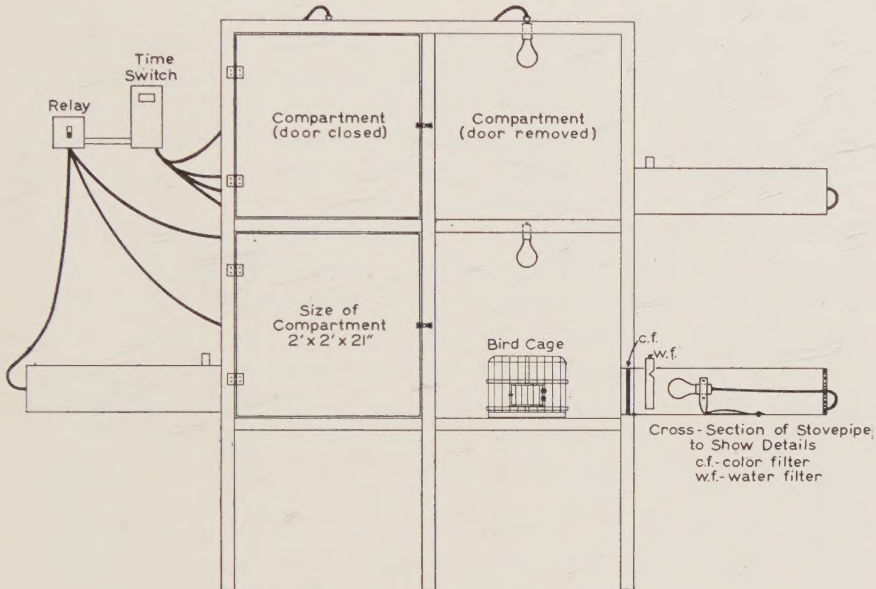


FIG. 1. Sketch of the light-tight cabinet used.

own work, I found that sharper higher peaks were obtained in *P. cathe-merium* infections under ordinary conditions if the smears were made at 4, 6 and 8 P M rather than at 3, 5, 7 and 9 P M.) In this connection, it must be remembered that the peak of segmenters precedes the actual peak of schizogony by a few hours. The procedure which I found best for my work was to make smears at 2- or 4-hour intervals, over a consecutive period of 72 hours (sometimes less), to examine 200–300 parasites (50–100 in the few cases where parasites were scarce) on each smear and to consider a segmenter to be a parasite with 8 (in a few instances 5; see Fig. 6) or more nuclei.

The Effect of Light on Periodicity

To determine whether light influences periodicity and whether it acts through the eyes or through the body surface of the canary, the follow-

ing experiment was devised. Twenty-four canaries were placed in groups of 8 in three compartments of the light-tight cabinet under the following conditions. A 40-watt bulb furnished the illumination for the light periods in all compartments. Compartment A had continuous light with food constantly present. Compartment B had continuous light with food present during only 12 hours of each 24-hour period (10 A M–10 P M). Compartment C had 12-hour periods of light with food (10 P M–10 A M) alternating with 12-hour periods of darkness without food. These are essentially controls, but it is to be noted that their light (10 P M–10 A M) and dark (10 A M–10 P M) periods were a reversal of normal conditions.

On the 10th day of the experiment birds 1C and 2C in compartment A, 3C and 4C (this bird died prematurely) in compartment B and 5C–8C in compartment C were inoculated with the "C" strain. Three days afterwards, these birds were killed and their blood inoculated intravenously into the 16 other birds, as indicated in Table I. In the meantime,

TABLE I.—*Cap experiment of 18 days duration, showing the conditions of light, darkness, food and wearing of cap. From 15th through 18th day of experiment, i.e., beginning 2nd day after birds were infected, slides were made for 4 days at approximately 2-hourly intervals (See Figs. 2 and 3)*

Bird number	Group	Conditions in compartment			Infected on 13th day of Exper. from :
		Light during 18 days	Food during 18 days	Caps 10AM–10PM 5th to 18th day	
9C	A b	Continuous	Continuous	No	1C, 2C ¹
10C	" b	"	"	"	5C–8C ²
15C	" c	"	"	Yes	1C, 2C
16C	" c	"	"	"	1C, 2C
17C	" c	"	"	"	5C–8C
18C	" c	"	"	"	5C–8C
11C	B b	"	10AM–10PM	No	3C ³
12C	" b	"	"	"	5C–8C
19C	" c	"	"	Yes	3C
20C	" c	"	"	"	3C
21C	" c	"	"	"	5C–8C
22C	" c	"	"	"	5C–8C
13C	C b	10PM–10AM	10PM–10AM	No, but in dark 10AM–10PM	1C, 2C
14C	" b	"	"	"	5C–8C
23C	" c	"	"	"	3C
24C	" c	"	"	"	5C–8C

¹ 1C, 2C, pooled blood from 2 birds which had been kept in continuous light with continuous food for 13 days and which 3 days before being killed were inoculated with the "C" strain.

² 5C–8C, pooled blood from 4 birds which had been kept in light with food for 12 hours and dark without food for 12 hours for 13 days and which 3 days before being killed were inoculated with the "C" strain.

³ 3C, blood from 1 bird which had been kept in continuous light with food 10 A M–10 P M for 13 days and which 3 days before it was killed was inoculated with the "C" strain.

on the 5th day of the experiment, four birds in compartment A (15C–18C) and four birds in compartment B (19C–22C) were made to wear caps in the interval 10 P M–10 A M each day thereafter until the end of the experiment whereas the remaining birds in compartment A (9C, 10C), in compartment B (11C, 12C) and in compartment C (13C, 14C,

23C, 24C) were left under the conditions existing in their special compartments. The caps were made of black cloth with a beak slit for unhampered breathing and were fastened at the back of the head by a purse string arrangement. Although a bird struggled for a time after a cap was put on, it suffered no undue inconvenience and sooner or later settled to rest and remained so until disturbed. Smears of birds 9C through 24C were begun on the 15th day of the experiment, the parasites in the interim becoming adjusted to the conditions of their hosts. The infections in these birds were very heavy and of short duration with two-thirds of the birds having a definite number crisis during the period when smears were being made.

Figs. 2 and 3 show the results obtained in this experiment on the percentage of parasites occurring with 5 or more nuclei. The birds in the first 3 graphs of Fig. 2 were inoculated from birds 1C and 2C whose infection showed a somewhat disrupted periodicity due to the continuous light to which the birds were subjected. Bird 9C (not capped) exhibits no asexual periodicity of its malaria parasites, whereas bird 16C (capped) shows a definite asexual periodicity with segmentation peaks occurring at the end of the period when no cap was worn. Bird 15C which was also capped died prematurely. Bird 13C under control conditions, shows a slight asexual periodicity, but apparently this bird was unable during the course of the experiment to resolve the parasites into a brood segmenting with a clear-cut periodic rhythm. The birds in the last 3 graphs of Fig. 2 were inoculated from bird 3C whose infection showed a slightly disrupted periodicity. Bird 11C shows a slight periodicity, whereas the time of peak segmentation differs by 12 hours in birds 19C and 23C. Such a condition indicates that the periodicity of the parasites in each of these birds had become readjusted to sporulate at the beginning of the dark period and was, therefore, behaving normally with respect to light and dark. The birds in Fig. 3 received parasites from 5C-8C (normal periodicity) and showed a relatively high percentage of segmenters at the beginning of their infections. In birds 14C and 24C, under control conditions, the periodicity is maintained with the peaks appearing at the beginning of the period of darkness. In bird 10C, the periodicity is progressively lost. Bird 12C shows a gradual reduction in peak magnitude and a progressive shift of peak time from 10 A M to 6 P M near the end of the period of feeding. Birds 17C, 18C, 21C and 22C, which wore caps 10 P M-10 A M, all show a definite shift of segmentation from the 10 A M hour (of the injected parasites) toward the 10 P M hour. Although the segmenters were never present in large numbers, the constant direction of the shift of the peak time, the similar response in all four birds, and the presence of a periodicity seem to confirm the opinion that the parasites in these birds were arranging themselves into a cycle

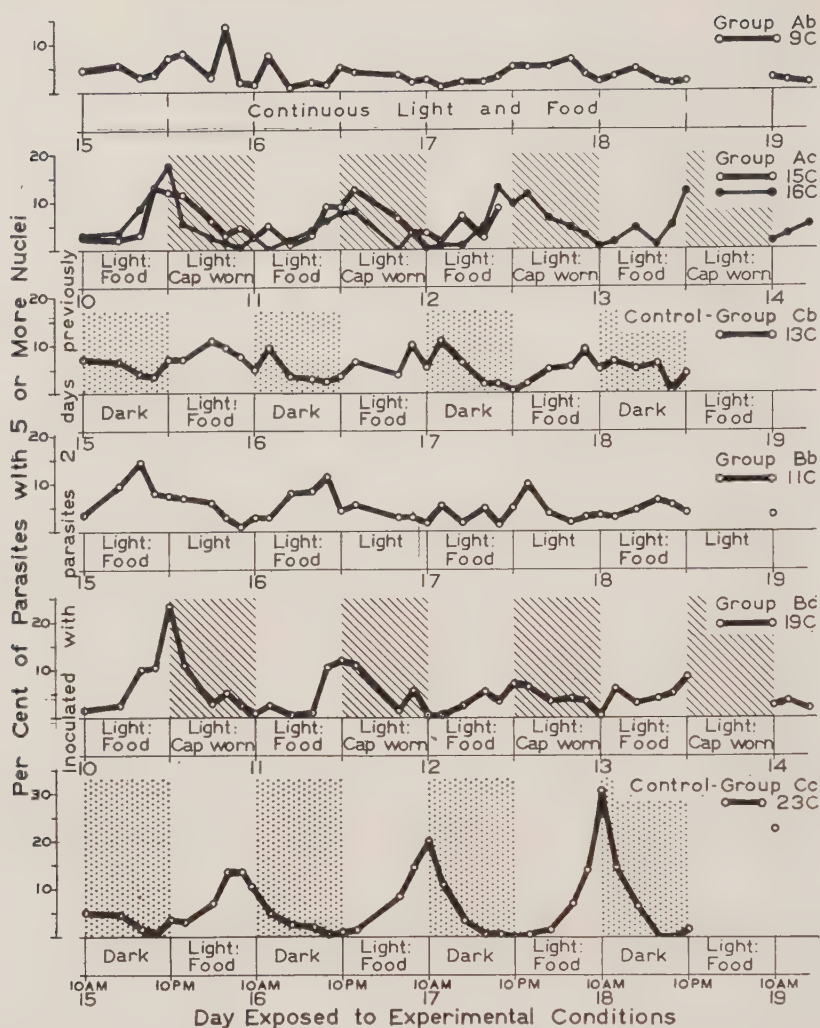


FIG. 2. The effect of various host conditions on the asexual reproductive rhythm of the "C" strain of *P. cathemerium*. Stippled areas indicate the host period of darkness, cross-hatched areas the period (relative darkness) when certain canaries wore caps.

of synchronous reproduction with the peak time at the beginning of the period in which they wore caps.

In summary, all of the capped birds (15C, 16C, 17C, 18C, 19C, 21C and 22C) responded in the same way regardless of the other conditions to which they were exposed or the source of the parasites used for inoculation. In some birds the time when segmenting parasites most frequently appeared definitely shifted, in others synchronicity was reconstructed from a condition in which none existed. In every case as the

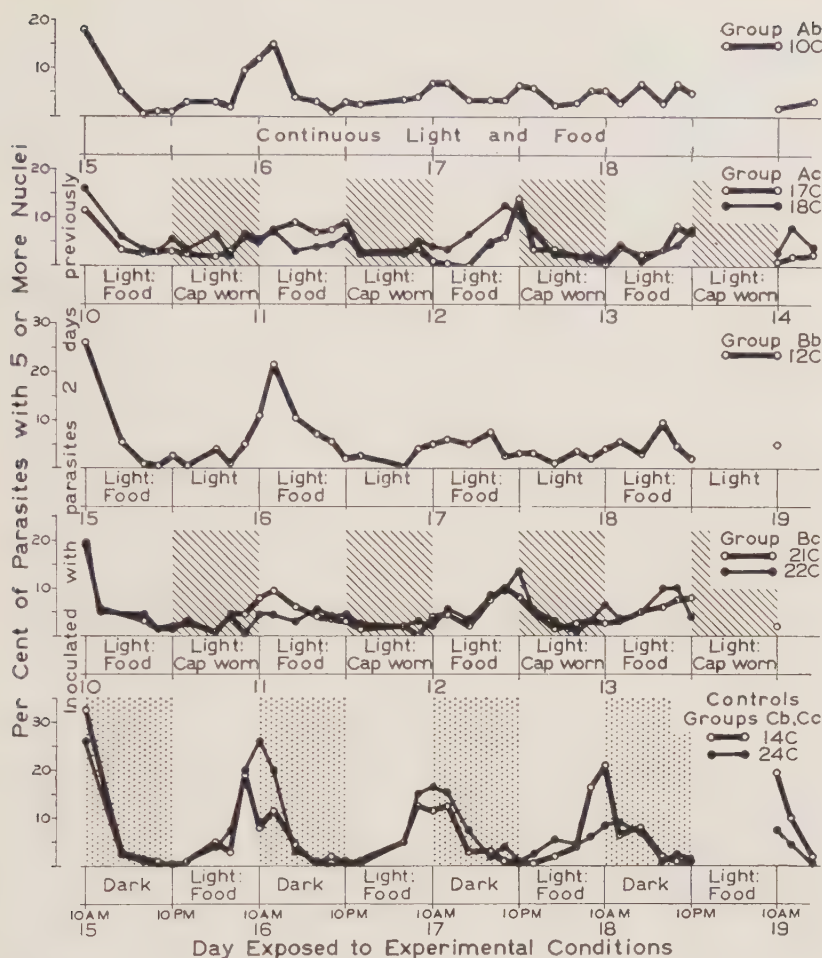


FIG. 3. The effect of various host conditions on the asexual reproductive rhythm of the "C" strain of *P. cathemerium*. Stippled areas indicate the host period of darkness, cross-hatched areas the period (relative darkness) when certain canaries wore caps.

infection progressed, the time of peak segmentation occurred near the beginning of the period when caps were worn. The influence of light on the general body surface seems ruled out as a factor in periodicity. The part played by the eyes in this experiment may be through a direct effect of light or through an indirect effect on the ability to feed or to rest.

The Effect of Environmental Temperature on Periodicity

Because the periodicity of the asexual cycle of avian malaria was found to be disturbed during a heat wave in the summer of 1934, some experiments on temperature changes were carried out.

In the first experiment, groups of birds were placed in the light-tight cabinet. Three of these groups were subjected to a temperature of 37°C by means of a hot plate during the dark foodless period (10 A M–10 P M) and were subjected to a temperature of between 24°C – 29°C during the light-food period (10 P M–10 A M). The control group D was subjected to a temperature of 24°C – 29°C during the dark foodless periods (10 A M–10 P M) as well as during the light-food periods (10 P M–10 A M). (It will be noted that all of the birds, including the bird from which the infection of "N" strain was obtained, were on a light and dark schedule the opposite of normal.) Of these groups, A and B were infected 3 and 1 day later, respectively, whereas group C was infected 3 days later with parasites from 1N which had already been subjected to high temperature for 4 days. Judging by the time, height

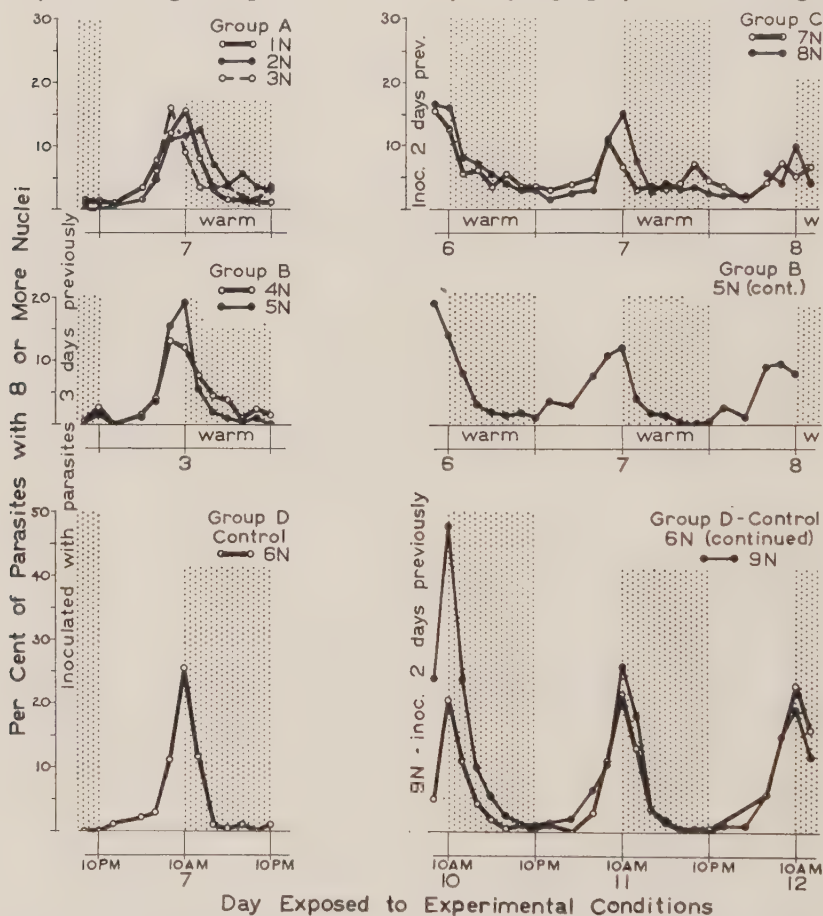


FIG. 4. The effect during the canary's period of darkness of high environmental temperatures on the asexual reproductive rhythm of the "N" strain of *P. cathemerium*. Darkness represented by stippled areas.

and spread of the peaks and the number of segmenters found in the periods between the peaks in groups A, B and C as compared with birds 6N and 9N in control group D in Fig. 4, the high temperature produces a disturbance in the periodic rhythm of segmentation of the parasites which is commensurate with the length of exposure. Thus, the parasites in birds 4N and 5N of group B which were subjected to high temperature for only a short time (2 days) before observations were made, show less disturbance in the cycle than those in either 1N, 2N and 3N of group A, the birds of which had been subjected to high temperature for a longer period (7 days) or those in 7N or 8N of group C, the birds of which and the parasites of which had both been subjected to high temperature for longer periods (6 days).

In the second experiment four groups of birds were subjected to the "R" strain and to more extreme temperature conditions which were made possible by an incubator and ice chest. The temperatures in the lighted half of the incubator were somewhat higher ($31\text{--}43^{\circ}\text{C}$) than in the darkened half ($31\text{--}38^{\circ}\text{C}$). The temperature in the ice chest varied from $14.4\text{--}20^{\circ}\text{C}$, but usually ranged around 15°C . Food was present during the light period for all birds. Birds 1R and 2R in group A were subjected to high temperature during the light period, and to cold during the dark period, and the procedure was reversed for birds 3R and 4R in group B. Birds 5R and 6R in group D were subjected to continuous high temperature whereas 7R and 8R in group E were subjected to continuous cold. The control bird 9R in group C was subjected to continuous normal room temperature. The "R" strain of parasites used for inoculation was originally obtained by pooling the blood from two birds which differed in day and night schedules and whose parasites, therefore, segmented at different times, so that the power to readjust the periodic rhythm could be studied. In birds under normal conditions, segmentation quickly reverts to a synchronous periodicity (see 9R, Fig. 5). These parasites were inoculated into one of the foregoing groups after they had been for 2 days in a cage mate. Thus, they had a sojourn in a bird under similar experimental conditions before they were injected into the test bird.

Although the infections in this series were low grade, a comparison of the per cent of parasites with 8 or more nuclei between the various groups revealed that the usual synchronism of the asexual cycle is disturbed by periodic changes in temperature (groups A and B) and also by continuous high temperature (Group D) or continuous cold (Group E). Except in bird 2R, however, the synchronicity was not affected to the degree that it was in the "N" strain (Fig. 4).

In a third experiment with the "D" strain performed in a manner similar to the second experiment in this series, the greatest deviation from

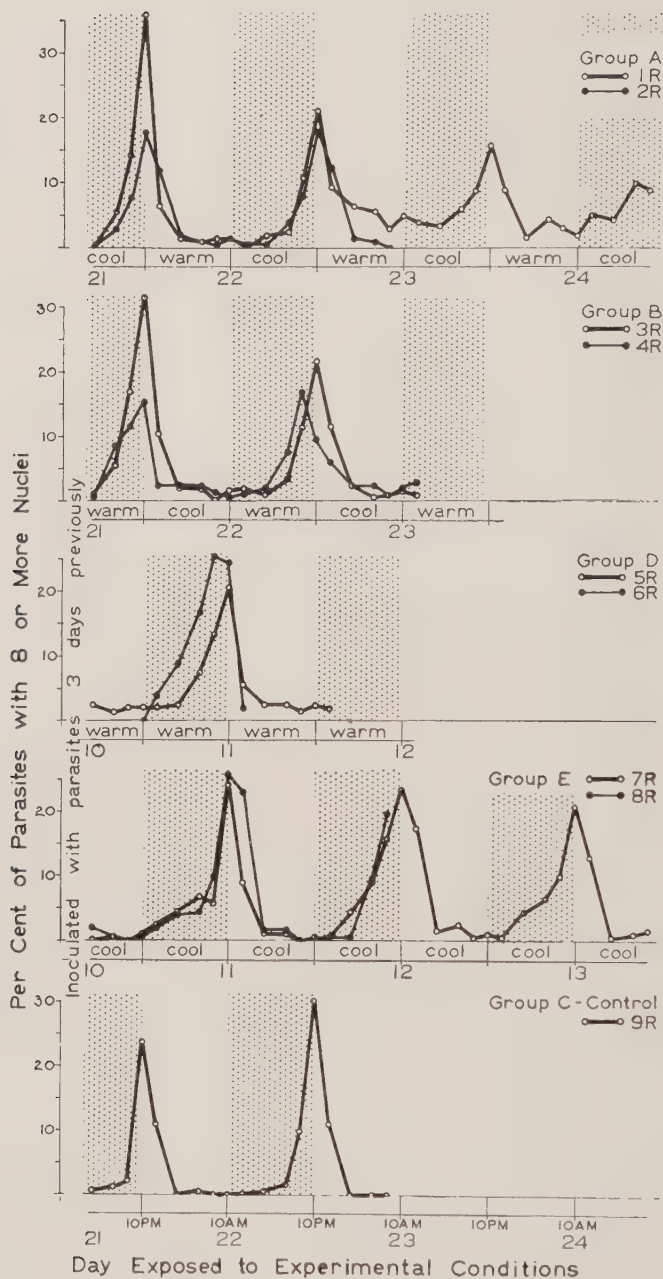


FIG. 5. The effect produced on the asexual reproductive rhythm of the "R" strain of *P. relictum* var. *matutinum* by exposing the canary host to unusual combinations of environmental temperatures. The stippled areas represent the period of darkness.

the control was obtained with group B birds subjected to incubator temperatures during the period of darkness and to ice chest temperatures during the light period while the other groups exhibited synchronous reproduction but with altered time in the peak of segmentation.

These three experiments seem to indicate that high environmental temperatures alternating with room or ice chest temperatures caused a significant disturbance in the asexual periodicity of these avian malarias. Moreover, since the most significant deviation in the cycle (see especially Fig. 4, 7N and Fig. 5, 2R) occurred when the temperature was high during the young trophozoite stage, it would seem that this portion of the life history of the parasite is more susceptible to increased atmospheric temperature surrounding the host than other portions. Whether the effect is produced directly on the growth rate of the parasite or indirectly through some other process or processes is not evident from the results obtained. Continuous high or cold temperatures failed to produce as pronounced results although some disturbance is noted in 5R and 6R. Until further data are obtained we assume that normal conditions of body temperature can be more nearly approximated in constant heat or cold than in alternations of the two.

The Effect of Activity and Feeding on Periodicity

The importance of feeding (with its concomitant muscular activity and resultant digestive and assimilative phenomena) and of rest in the control of the reproductive rhythm of the parasites was tested by alternating 12-hour periods of high and low intensity illumination and adjusting the time of feeding so that some birds (subgroup "a") were allowed to feed only during the period of higher intensity light while others (subgroup "b") were allowed to feed only during the hours of lower intensity light. The intensity of light was varied as follows: In compartment A which represented the greatest range of light conditions, the center of the cage received 105 foot-candles of light from 100-watt bulb 10 A M–10 P M and 1.5 foot-candles of light from a 7½-watt bulb from 10 P M–10 A M. In compartment B, the alternate intensities for the same time intervals were 41 foot-candles of light from a 60-watt bulb and 4.5 foot-candles of light from a 15-watt bulb. Similarly, in compartment C, the alternate intensities were 20 foot-candles of light from a 40-watt bulb and 7.3 foot-candles of light from a 25-watt bulb. The birds were adapted to the experimental conditions for 14 days before infection except in subgroups Ab and Ca (Fig. 6) where the birds originally intended for the experiment died prematurely and others had to be substituted. The "N" strain for this experiment came from the pooled blood of 2 birds, one of which had been kept under ordinary animal room conditions with normal periods of daylight and darkness and the other

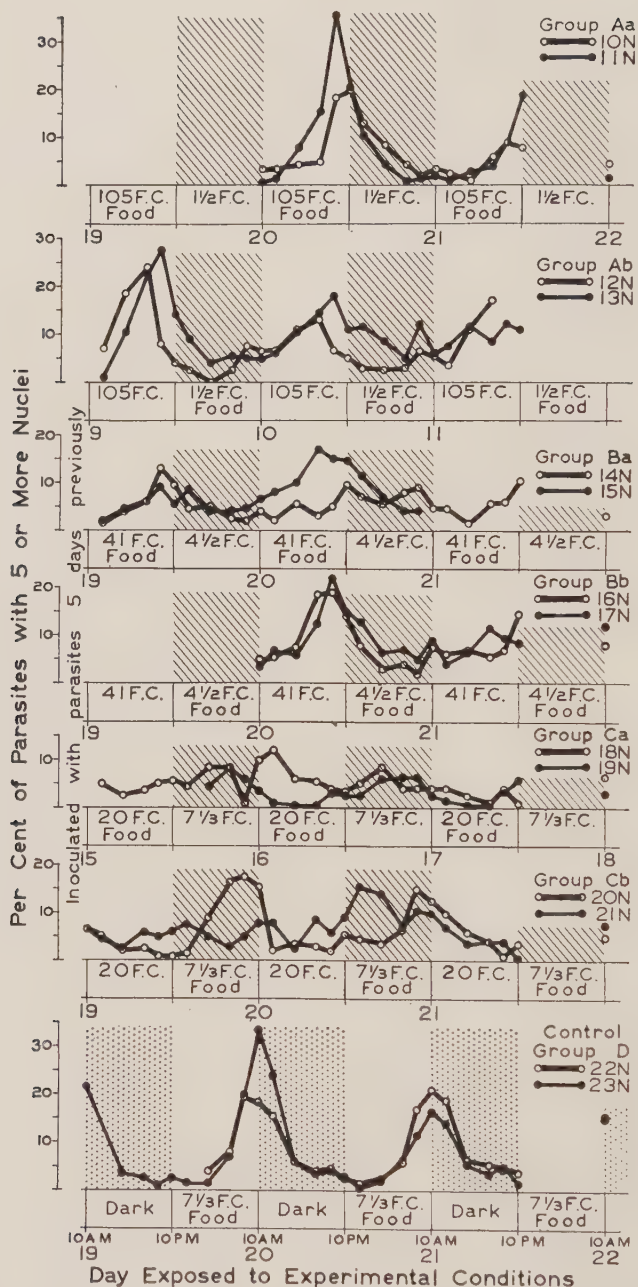


FIG. 6. The effect on the asexual reproductive rhythm of the "N" strain of *P. cathemerium* of exposure of the canary to alternate periods of different intensity illumination. Cross-hatched areas represent period of lower intensity illumination or relative darkness. The stippled areas represent the period of darkness for the control birds.

of which had been kept in the light-tight cabinet on a reversed schedule. The 12 experimental birds and 2 controls were inoculated intramuscularly and 5 days later blood smears were made at frequent intervals to cover at least about 2 complete asexual cycles of the parasite. These data will be found in Fig. 6.

The periodicity obtained in the control birds 22N and 23N is slightly less regular than that obtained in the temperature experiment with this strain of parasites, but the peaks of segmentation occur, as would be expected, at or near the beginning of the period of darkness. The birds in group Aa, (10N, 11N) which were subjected to 105 foot-candles with food and 1.5 foot-candles without food show an asexual periodicity almost as marked as in the controls with the peaks appearing at or near the beginning of the period of lower light intensity or relative darkness. The birds in subgroup Ab (12N, 13N) which were fed during the period of lower intensity light also show definite peaks in segmentation at the beginning of the period of lower light intensity, but the peaks are not so sharp. The birds in group Ba (14N, 15N) under more nearly equal light intensities consisting of alternate periods of 41 foot-candles with food and 4.5 foot-candles without food, and the birds in group Bb (16N, 17N) which were fed during the low light intensity also show a major segmentation near the beginning of the period of lower light intensity, which, however, is not nearly so marked as that in the controls. Thus, the data for these 4 groups indicate that the period of lower intensity of light exerts a greater influence than the time of feeding. The birds in compartment C, where the light intensity alternated from 20 to 7.3 foot-candles, show results more nearly like those reported for continuous light. In subgroup Ca (18N, 19N) there is almost a complete disruption of the synchronism, with segmenters found on nearly every slide and the peaks with one exception less than 10 per cent. The birds in subgroup Cb (20N, 21N) also show a disruption of the customary periodicity of the malaria parasites. Bird 20N shows well-defined peaks of segmentation occurring at 8 A. M. on successive days near the *end of the period of food and lower intensity light*. Peaks occur in 21N at almost 12-hour intervals, possibly reflecting the two broods of parasites inoculated.

During the course of this experiment as blood smears were made, the number of sleeping birds in each cage was recorded in order to obtain an idea of the activity of the birds. In compartment A where the light intensity differed most, the birds slept only during the period of low intensity light although birds of subgroup "b" also had to feed in this period. If the period of rest is taken as our focal point, regardless of whether or not it was interspersed with feeding, we find the peak of segmentation related to the rest period, or probably, as Boughton (1933)

suggests for coccidia, to a previous rest period. Birds in group B showed similar periods of rest (an occasional bird was found asleep during the period of high intensity illumination) and again the data point to a relationship with the period of rest or sleep. Birds in group C, where the light was more nearly continuous, were found asleep at various times, but predominantly during the period when no food was allowed them regardless of the light intensity. The period of rest, therefore, in these birds became related to the period of feeding. In no cage did as many as 100 per cent of the observations show birds asleep. During the period of study blood smears were being made frequently during the day. This constant disturbing factor, especially in the presence of continuous light, must certainly have produced this effect, at least in the birds in subgroups "a." In birds of subgroups "b" this disturbing factor would also act but the necessity for feeding in the low light period would of itself decrease the percentages of sleep observations in this period.

A second experiment, using the "C" strain of *P. cathemerium* performed under the same conditions and procedure, gave confirmatory results.

Since the "R" strain of *P. relictum* var. *matutinum* shows such a markedly periodic and synchronous asexual reproductive activity and since it sporulates normally at the beginning of the light period instead of at the beginning of the dark period as does *P. cathemerium*, it was tested under the same conditions and procedure outlined above. The infections were low grade and transitory, typical of the "R" strain, and in some cases only enough parasites to make one complete cycle could be obtained, but the results, shown in Fig. 7, are surprisingly similar to those met with in the "N" strain.

In these experiments where light and dark are minimized and activity and feeding are accentuated two broods of parasites were injected into each bird, one of which segmented in the evening hours and the other in the morning hours. In most of the birds that brood became dominant whose sporulation time had the same relation to the rest period that the sporulation time in normal birds has to dark and light. This is extremely interesting in view of that fact that the brood of the "N" strain dominated whose sporulation took place at the beginning of the rest period (10N, 11N, 16N, 17N, 19N, 20N) and the brood of the "R" strain dominated whose sporulation took place at the beginning of the active period (10R, 11R, 13R, 14R, 17R, 18R). In a few birds, however, both broods tended to be maintained (21N, 15R), and in a few others sporulation of the two broods seemed to shift to an intermediate time (12N, 13N, 14N, 15N, 18N, 12R, 16R). These data are consistent in indicating that the rest period is far more important than feeding in the periodic cycle of reproduction and that feeding only becomes important when it

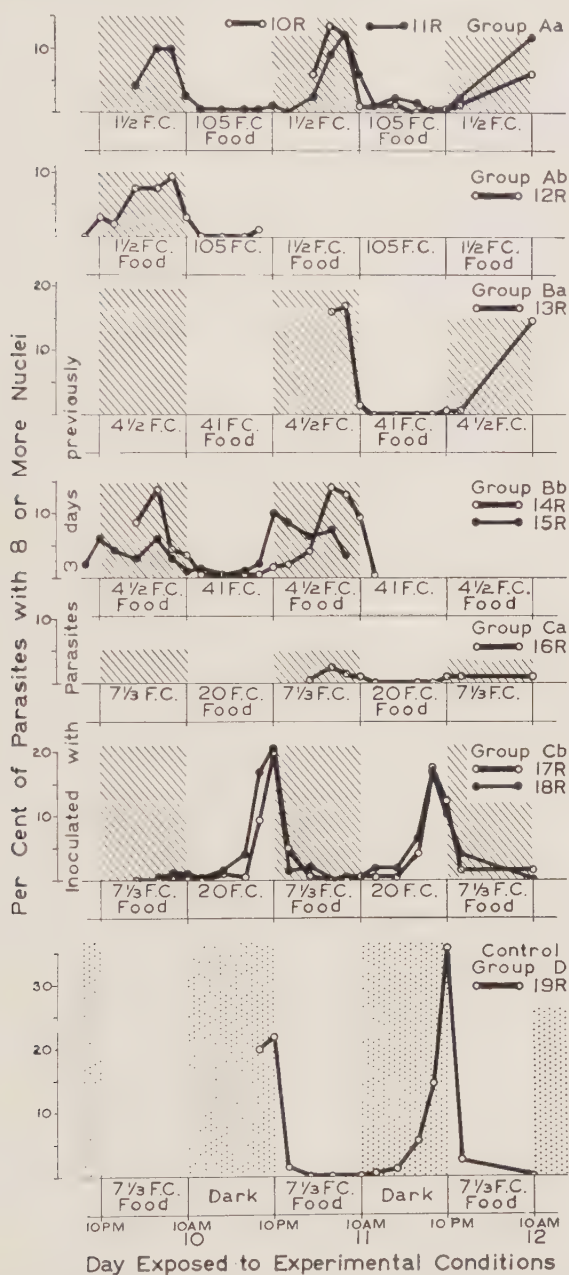


FIG. 7. The effect on the asexual reproductive rhythm of the "R" strain of *P. relictum* var. *matulinum* of exposure of the canary to alternate periods of illumination of different intensity.

interrupts or conflicts with the rest period. The next experiment bears out this conclusion.

In this experiment, 2 control and 2 experimental birds were exposed to light 11 P M–9 A M. During this time the control birds were allowed to feed at will, but the experimental birds were given no food (water was present) except in the last hour of the light period (8 A M–9 A M). At 9 A M both groups of birds were put in the dark except at intervals (roughly from 11:00 to 11:15 A M, 1:30 to 1:45 P M, 4:30 to 4:45 P M and 9:55 to 10:10 P M). During these light intervals, only the experimental birds were allowed to feed. After 12 days of acclimatization, the birds were inoculated with the "C" strain from two birds, and 4 days later blood smears were made and studied. Since the parasites in both groups showed cycles of reproduction entirely similar to that usually seen with the "C" strain of parasites, no graphs of the results are presented. The peak of segmentation occurred at the end of the light period regardless of the time food was taken.

DISCUSSION

A study of the periodicity in malaria is complicated by several variable factors. In quartan simian malaria, W. H. and L. G. Taliaferro (1934a) have shown that the crisis of the malaria infection may at times delay the asexual cycle by as much as a whole day. Boyd and Allen (1934) have also shown that as the acute infection proceeds the mean size of the segmenting parasite and the number of merozoites produced by it both diminish as the parasite number increases until the crisis is reached and then slowly return to their former level. Therefore, we have attempted to study only the acute rise of the infections herein presented. That this was not possible in every case is due in part to differences in the type of malaria infection produced by the various species, to the route of inoculation and to the dosage. Furthermore, to minimize loss of blood and disturbance to the bird, very small smears were made as quickly and gently as possible.

Boyd (1933) believed that host feeding is an important factor in periodicity, but the time of sleep may be the important factor since he did not separate the two. In all cases except one he used continuous light. According to our observations, however, birds do not tend to rest well in the light, especially when blood smears are being made at frequent intervals. In one case where continuous light was not present, his birds were force-fed during the dark period.

It is known from Szymanski's work (1920) that the canary is an "optical" animal, restricting its activity to the hours of exposure to light. It is also known that birds have a diurnal temperature cycle which is high during the light hours and lowest during the dark or rest period (L. G. Simpson and Galbraith, 1905, or Kendleigh and Baldwin, 1928).

This has been confirmed for the canary by Huff (unpublished). The synchronism of asexual reproduction of avian malaria may depend on the body temperature of the host. Huff has found that canary temperatures may vary 6° C between the daily maximum and the daily minimum. Critical temperatures for reproduction are a well-known phenomenon. For example, the oyster, *Ostrea virginica*, will not spawn in Barnegat Bay, N. J., until the water temperature has reached 20° C. Even this temperature is more effective if reached suddenly than when very gradually (Nelson, 1928). If we assume that some stage in the avian reproductive cycle or some phase of the parasite's metabolism has a critical temperature that is reached once daily in the canary, we need only further assume that a definite rest period is necessary to obtain a temperature differential great enough for this effect to be expressed. On this basis the results presented here all fall into line. Any disturbance of the rest period could then alter or shift the time of peak segmentation. Environmental temperatures surrounding a somewhat poikilothermic animal (the canary) could also modify the usual cycle of reproduction. Other factors, as time of feeding, when not interfering with the rest period would not affect the cycle. Light itself would exert a direct effect only in stimulating activity in the host. There seems to be, however, a special relationship in the temperature experiments. When high environmental temperatures were alternated with low temperatures, marked disturbance of the synchronism was observed only when the high temperatures were present during a certain stage of the parasite (young trophozoite). When the high atmospheric temperatures occurred during the other half-day no such marked disturbance of the cycle was obtained.

Can species differences also fit into such an hypothesis? For example, the "R" strain segments at a time which is antipodal to that seen in the "C" strain. We can assume either that altogether different factors act on different stages of the life cycle, that different factors act on a similar stage in the reproductive cycle or that the same factor acts on the same stage of the parasite, but that the critical level of action is different. The last alternative would furnish the situation explainable on the basis of body temperature. In support of this contention we can refer to the temperature experiments in which the most marked deviations of reproductive rhythm occurred (in those birds infected with either "R" or "C" strain parasites) when high environmental temperatures prevailed during that stage of the parasite cycle when almost all the forms were young trophozoites. Both Boyd (malaria) and Boughton, Atchley and Eskridge, 1935 (coccidia) found that lengthening the bird's day to an artificial day of 28 or 29 hours respectively resulted in a lengthening of the time between the peaks of segmenters or oöcysts to correspond to the artificial days. They also noted, however, that the

time of the peak had shifted from the evening hours to the morning hours. Since we do not know the details of the host response to these conditions, we can only postulate that either some of the parasites in the clone have slightly different critical levels which are favored or inhibited by the new conditions or that with the loss of the original critical factors another set comes into play. All other deviations, such as parasites with 30-, 36- and 48-hour cycles of reproduction can be explained on a genetical basis with or without a synchronizing factor or by assuming that the canary is an abnormal host. There is no reason for assuming that every strain of malaria should have an asexual periodicity. A counterpart is observed in filarial studies where both synchronous and asynchronous strains exist. In filariasis, the presence or absence of a cycle has been related to the biting habits of the insect vector. In malaria, since the presence of mature gametocytes shows a periodicity which is related to the asexual rhythm (Gambrell, 1937) we might suspect that the biting habits of the natural vectors of the various species of avian plasmodia are related to these periodic phenomena. The specific natural vectors of the avian malarias are not known, however.

It is true that the proof for this hypothesis is not yet conclusive, but it does make a working theory which seems at present to explain all the known major modifications of the periodicity of avian malaria. Only further study will demonstrate its true value. One point, however, requires emphasis. The young trophozoite seems to be the stage most susceptible to experimental influences and probably plays the important role in orienting the synchronous rhythm. There is also the possibility that what we have designated as critical body temperature may actually be some other physical state or physiological process which occurs simultaneously and synchronously with the changes in temperature and which is likewise affected by changes in environmental temperature, period of rest, etc., as we have assumed for body temperature.

SUMMARY

1. A study of the experimental modification of the asexual periodicity of avian malaria was conducted on three strains of *P. cathemerium* ("C," "D" and "N") and on one strain of *P. relictum* var. *matutinum* ("R"). One of the strains ("N") was obtained by single-parasite isolation.
2. The synchronous periodicity of reproduction of avian malaria is affected by alternate 12-hour periods of high and low temperature.
3. It is affected by light itself—active through the eyes and not through the body surface—if of sufficient intensity to cause the host to be active.
4. As a corollary to (2), it is associated with rest or sleep which seem to be important orienting factors.

5. It is not affected by host feeding of the type devised in these experiments.

6. It is considered possible that the host may furnish a set of critical temperature conditions which orients the time of segmentation. In such an hypothesis, the period of rest becomes important in that it makes possible the temperature differential necessary for the appearance of the critical value.

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